# BACTERIA IN ICE, ESPECIALLY IN THEIR RELATION TO TYPHOID FEVER.

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It has always been supposed that the freezing of water, like its distillation, excludes contained impurities; but, as I think, can be clearly shown, the idea is erroneous. True, gross impurities and much organic and mineral water are thrown out by freezing; but the bacteria, which probably are, in most cases, the only really harmful matters held in natural waters, are retained. More than this, only a certain portion of these bacteria are killed by low temperature. This is not surprising when looked at in the light of the long-known fact that higher organized forms of animal life, such as frogs and fish, have retained their vitality after being pent up in ice for considerable periods.

Ice, like water, may look pure and clear, though literally swarming with bacteria. As shown by Prudden, one and one-half millions of micro-organisms may be added to a tablespoonful of perfectly clear distilled water without appreciably altering its limpidity when held up in a test tube to a strong light. Bubbly or "snow" ice contains often an immensely greater number of bacteria than that which is clear. Here, however, the opaqueness is not due to the micro-organisms present, but rather the bacteria collect there as the result of the operation of capillarity and presence of air. To so great an extent does the idea that ice must be pure prevail, that it is a common thing for people who would be horrified at the idea of drinking from a known filthy body of water to much enjoy drinking melted ice from the same source, though the water probably contains almost if not quite as much danger after as before freezing.

Three years ago I made, during a period of about six months, a series of microscopical and biological analyses of the ice supplied to

the general market. I was led to these investigations by the discovery I had made in an endeavor to ascertain the cause of three cases of typhoid fever in a family living in the suburbs of Pittsburgh. Careful analysis of the drinking water (piped from a spring on the hillside, fifty feet higher than, and three hundred and fifty feet from, the house) failed to show anything suspicious. The patients did not remember having drunk any water away from home for three weeks prior to the onset of the disease, not having been off the premises for more than an hour or two at a time during those three weeks. A case of typhoid fever had, however, occurred in the family about a year previous to the occurrence of the cases mentioned, the patient having been brought home from a visit in the incipiency of the disease. A careful search into the matter finally led me to examine the ice, which had been used freely, with the result of finding in it enormous numbers of bacteria, especially of fecal and putrefactive species. This was considered evidence enough that here was the source of the trouble, even without the corroboratory evidence of my discovery, in the ice, of Eberth's bacillus of typhoid fever. The ice had been taken from a large pond or lakelet horribly polluted with surface water, and receiving the overflow of a cess-pool about twenty feet away from the water's edge on one bank. The bulk of the ice from this pond was marketed, innocently enough on the part of the owner; for, though he knew the pond to be filthy in hot weather, he thought freezing purified the whole thing, especially the portion of the water that formed ice.

The fact that this ice was marketed led me to think that some of the market ice from other sources might also be contaminated. With the object of ascertaing to what extent this might be the case, I examined 20 samples of ice from each of seven different companies. Each of the samples from the respective companies were procured on twenty different days at intervals of a week. This plan was adopted in order to get the sample from different blocks of ice, so as to assure a fair average of purity; for, as might be supposed, there is considerable variation in the distribution of bacteria through ice from the same body of water. In collecting these samples, ice containing gross foreign matter, as leaves, grass,

mud, etc., was rejected, for the reason that such ice is not used in food or drink by the consumer, and is not delivered by most dealers. Besides this, dirty ice usually represents but a very small proportion of the crop of any given ice field.

Before giving the results of my investigations, I shall say a few words explanatory of the methods and the results to be gotten from biological analysis of drinking water.

Modern science has shown, that apart from mineral poisons and such unlikely contaminations, the greatest danger in a drinking water arises from the various micro-organisms that it may contain. The probable presence of these may be indicated by chemical analysis; but it is only by biological analysis that anything like a satisfactory solution of the problem of the suitability of any given sample of water for portable purposes may be determined. To put a few drops of water to be examined, or a portion of the sediment therefrom, under the microscope, and attempt to get by any amount of observation the relative or actual purity of the water, would result in partial if not complete failure. But by biological analysis painstakingly carried out in all its details, and with the microscope as an essential, the investigator is enabled to determine with comparative accuracy the presence of living bacteria, the number of their species, the number of individuals, their relation to disease, and their effect on the animal organism and, by inference and analogy, their effects on the human economy. Further, this method of analysis enables us to determine the value of any given process of filtration or other method of purification.

Biological analysis consists of certain technical processes on the careful carrying out of the details of which depends the accuracy of the results obtained. As applied to water, the method consists in the admixture of a definite quantity of water to be analyzed, say 1 c.c., with a convenient quantity of warm (and consequently fluid) sterilized, nutrient gelatine in a test-tube. This mixture is next poured out on a sterilized glass plate, setting level on a cold marble slab.

A glass dish filled with icewater and covered with a sheet of plate glass, the whole supported on a tripod having screw legs for levelling, is the apparatus usually used for this purpose; but I find a

marble slab of convenient size a very good and cheap substitute. The marble rapidly conducts away heat so that the gelatine hardens quickly. The marble will usually be cold enough in most any weather, but in summer, if necessary, it may be chilled with ice-water, and in winter it may be placed outside or near a window. Before using, it should be washed with corrosive sublimate solution (1 : 1000). To facilitate this sterilization, the surface of the slab should be highly polished.

On this (kept covered with a bell-glass) the gelatine soon cools, soldifying as it does so, thus imprisoning each individual organism in the mixture at whatever part of the mixture it may be. When hardened, the glass plate, covered with the solidified gelatine, is transferred to a damp chamber,* then the whole thing set aside in a warm corner or in an incubator at 24° C.—not warmer, lest the gelatine be liquified. Now, having supplied nourishment, moisture, and a suitable temperature, the bacteria will proliferate, so that every living individual bacterium will soon be surrounded by a cluster of progeny. This cluster is called a colony, and as each colony is the progeny of one original organism from the 1 c.c. of water that we have added to the gelatine, evidently there must be just as many colonies scattered through the gelatine as there were organisms in that original 1 c.c. of water. As the colonies grow they soon become visible to the naked eye or a low power as a white or colored spot, and by counting the number of colonies on the gelatine plate we get the number of bacteria in the original 1 c.c. of water. Test-tubes may be inoculated from the colonies for the further study of the morphological and biological characteristics of each species of bacterium present, many species being indicated by the appearance alone of the colony.

Obviously biological analysis deals only with *living* bacteria—those which are capable of doing harm.

In the examination of ice the same methods of analysis are pursued, the only difference being that, as a preliminary, the ice has to be reduced to water, and that without contamination with other bacteria. This is accomplished by the following process, which, in

* A damp chamber is made by placing a sterilized glass bell in a glass dish, on the top of which is fitted a piece of filter paper, moistened with a 1: 1000 corrosive sublimate solution.

the main, is that of Prudden.* A small lump (about half an ounce) is selected from the sample to be examined, placed in a sterilized flask and washed well in boiled and cooled distilled water; then the lump is rinsed several times in its own meltings, and afterward melted down. Further examination is carried out as detailed above.

So much for the methods, now as to the results of the analyses.

Of the twenty samples of ice examined from each company, ten were clear and ten opaque. This was for the reason that "snow" ice contains, usually, many more bacteria than the transparent article. The result of the analyses may be seen from the following table of the—

AVERAGE NUMBER OF BACTERIA TO 1 C. C. OF MELTED ICE.

| | |
|---|---|
| Company A | 61. |
| Company B | 207. |
| Company C | 1,131. |
| Company D | 2,063. |
| Company E | 2,106. |
| Company F | 5,207. |
| Company G | 36,296. |
| Total | 47,071. |
| Average | 6,724.+ |
| Average omitting Company G | 1,795.+ |

By way of comparison, here is a table from recent investigations showing the—

RELATIVE PURITY OF MELTED ICE AND DRINKING WATER.

| | |
|---|---|
| Average number of bacteria in 1 c. c. of melted ice | 6,724. |
| Av'ge num. bac. in 1 c. c., etc., omitting that of Company G | 1,795. |
| " " " " of Pittsburgh faucet water | 7,021.† |
| " " " " " Allegheny City faucet water | 8,132.† |
| " " " " " Philadelphia (Schuylkill) faucet water | 9,674.† |
| " " " " " New York City (Croton) faucet water | 243.‡ |
| " " " " " water supplied to above four cities | 6,267. |

Now, as a small tumbler has a capacity of about 210 c. c., we have the following table:

*_Medical Record_, Vol. XXXI, 341.
† Jackson.
‡ Prudden.

| | | |
|---|---|---|
| Average number of bacteria in a tumblerful of melted ice | | 1,412,040 |
| Av'ge num. bac. in tum'ful, etc., omitting Company G | | 376,950 |
| " " " " " | Pittsburgh faucet water | 1,474,210 |
| " " " " " | Allegheny City faucet water | 1,707,720. |
| " " " " " | Philadelphia (Schuylkill) faucet water, | 2,031,540. |
| " " " " " | New York City (Croton) faucet water, | 53,430. |

It will be seen from the foregoing that, taking an average of all samples, ice when melted yields a drinking water but one-twelfth better, as regards the number of contained bacteria, than the average of drinking water as supplied to four large cities; and is much inferior in quality to the supply of New York City. But granting, for the moment, that the ice of Company G was much more polluted than commonly occurs, and for this reason omitting it, we see that the average of the ice from other sources shows approximately two-thirds less bacteria. Again, taking the average of ice from all sources examined: If a man were to take a tumbler half full of water, and, after purifying it effectually, were to add to it an equal quantity of melted ice of average purity (about half and half is probably the average proportion in an ordinary cooler), he would have a mixture containing in that one tumblerful one-quarter of a million bacteria. And under favorable conditions these could develop in forty-eight hours a progeny of 75,000,000,000 ! Of course the question arises here: What proportion, if any, of these bacteria are of pathogenic or disease producing species ?

Quite a large number of species were found in the aggregate, and in certain samples the number was comparatively enormous; in most instances the number of species and number of individuals retaining much the same ratio. The attempt was not made to classify *all* the species found, but in the ice of the first two companies tabulated only the so-called air, water and earth species were identified. In the ice from all the other companies fecal and putrefactive species were found in one or more samples. In the ice from one source—that of Company G — given in the table as containing an average 36,296 bacteria to the cubic centimeter, I isolated in a characteristic pure culture on potato a bacillus identical in every way with Eberth's bacillus of typhoid fever. This was in the ice taken from the pond referred to at the beginning of this paper as causing four cases of typhoid, and which was being freely delivered on the mar-

ket. Of course, as typhoid fever is apparently not inoculable in the lower animals, it is a difficult matter to be positive of the identity of *Bacillus typhosus* from morphological and biological characteristics alone; yet we do not know of any other bacillus that has these characteristics; and in this instance we have corroboratory evidence in the fact of cases having occurred in persons using the ice containg the germs. But I do not wish to lay so much stress on the finding of these bacteria in this one instance, as upon the fact that the large number of species and the large number of individuals found is very good evidence that the water had been polluted with sewage. It is not always possible to find *Bacillus typhosus*, even when present; but in such cases, as in the present instance, the number of living bacteria is itself evidence enough of the polluted and dangerous condition of the water. If sewage is present, the germ of typhoid fever must at times be there too; for it is now an accepted fact in medicine that typhoid fever is almost invariably produced by drinking sewage contaminated water. Of the seven companies' ice examined, some samples of that of three companies was found to contain species of bacteria commonly found in sewage; and, further, it was in these same samples that the largest number of bacteria was found.

A noteworthy fact in connection with the above mentioned cases of typhoid fever resulting from the use of polluted ice is, that the circumstance is quite conclusive clinical evidence that the bacteria of typhoid fever are capable of producing their specific disease after the water containing them has been frozen.

With regard to the significance of the presence of bacteria in a drinking water, whether from the ice added or the water itself, it is difficult to satisfactorily apply numerical data. Certain it is, however, that absolutely pure water contains no bacteria, and the farther any given water or ice departs from this the less likely it is to be fit for drinking purposes, and the more probable it becomes that it has been dangerously contaminated with sewage. Still, some species of bacteria existing in water are, with some degree of certainty, known to be practically harmless; so that in a general way it may be stated that a water may be drunk with comparative safety without the preliminary of filtering or boiling, if it contains not

more than 100 bacteria to the cubic centimeter, provided none of them are proven to be pathogenic. Koch endorses the view that fifty bacteria to the cubic centimeter is the limit. According to this, none of the samples of ice the examination of which is here reported are fit for drinking. But it must be remembered that more than fifty bacteria of certain innocent species present in water and derived from the air or earth, or naturally existing in water, may be present without rendering the water unwholesome to drink. It must be remembered, too, that the only safeguard about a numerical limit is the fact mentioned, that if a water is contaminated with sewage the numbers will be in direct ratio to the extent of contamination; so that, inversely, a small number of bacteria is reasonably certain evidence that the water or ice has not been contaminated with sewage. And by sewage is the only likely way for pathogenic bacteria to get into a body of water. But it must not be supposed from the statements above that a water containing even so small a number of bacteria as ten to the cubic centimeter would be safe to drink if the whole number were pathogenic bacteria, for it is likely that the number ingested do not bear any certain ratio to the degree of danger of infection. Bacteria multiply mostly by fission, or constant division of each mature individual into two. Thus they proliferate by geometrical progression, or continued multiplication by two. Into what kind of figures this leads is known by every school boy who has figured on the problem of the farrier who was to be paid one cent for the first nail, the price to be doubled for each successive nail up to thirty-two. It is not surprising then to state that a single bacterium may, under favorable circumstances, reproduce its kind to the extent of some billions in forty-eight hours. In view of this I think it is a justifiable statement to say that the number ingested may be very small indeed, yet the disease be produced. What bacteria lack in size they make up in numbers.

Now, practically, what have we to fear from a polluted ice supply?

Of all diseases due to a specific germ—a germ which is liable to get into our drinking water — typhoid fever leads. It is a well-known fact that the germs of this disease always, or nearly so, gain access to the human alimentary canal, where they multiply, by way

of drinking water. This being the case, it becomes desirable to know if any or all of the bacilli of this disease, when present in any body of water, are killed by freezing. To determine this point the following experiment was performed: To 200 cubic centimeters of sterilized faucet water was added a small quantity of a pure culture on potato of Eberth's *Bacillus typhosus*, which culture had been obtained from the discharges of a case of typhoid fever. An analysis by plate cultivation showed that in one cubic centimeter of this mixture there were approximately 8,800 of the bacilli. Now, ten cubic centimeters of this mixture, containing 88,000 bacilli, was placed in each of twenty sterilized test tubes. The mouths of the tubes were at once plugged with sterilized cotton wool, and then placed in a wire cage outside a window on a stone sill. As it was winter time, the thermometer outside ranging from $-5^\circ$ to $-20^\circ$ C., the water in the tubes was soon frozen solid, and remained so for two weeks. On one day when the thermometer outside indicated a rise in the temperatue to $+2^\circ$ C., the cage and tubes were placed in a tin bucket covered with a lid, and the whole set in a pan, the space between the pan and bucket being filled in with alternate layers of salt and finely broken ice. In this manner the water containing the bacilli in the tubes was kept frozen solid for two weeks. At the end of this time the tubes were brought in one by one, and as soon as the frozen mixture within them would all be melted, one cubic centimeter of it was analyzed by plate cultivation, in the manner already described. The average number of living bacilli remaining in each tube (containing ten cubic centimeters of the bacterial emulsion) was 43,740. Therefore as there were 88,000 bacilli per tube before freezing, we may say roughly that 50 per cent. were killed by the freezing process. As the conditions to which these germs were subjected was almost exactly those under which river water naturally freezes, this experiment I think proves *experimentally* that only a certain percentage of the bacilli of typhoid are killed by the freezing of water containing them. *Clinically*, this fact is evidenced by the instance cited of this disease resulting from contaminated ice.

Now, practically, the conclusion to be drawn from these data seems to me to be that any body of water contaminated with sewage

can never be depended on to yield a pure ice supply. If the water is contaminated with sewage it probably contains, at times at least, the bacilli of typhoid fever; and as 50 per cent. of these remain uninjured and active after freezing of the water, the conclusion is inevitable that there is danger in a polluted ice supply. For, practically, a purification to the extent of destroying one-half the typhoid bacilli present amounts to nothing, and for this reason: Typhoid bacilli, like other bacteria of the same class, grow by fission, or splitting of the mature organism into two; so that it takes but one division to make up a loss of 50 per cent. This fission, under favorable conditions, could probably take place in from fifteen minutes to an hour.

There are a number of things that would seem to question the practical value of this matter of bacteria in ice.

In the first place, it might be argued that all water contains bacteria. True, but no water not contaminated with sewage contains anything near the number of individual bacteria or the number of different species present in some of the samples, and certainly never any of the fecal and sewage species found. The evidence, therefore, is conclusive and beyond argument that some of the ice furnished to our markets actually is diluted sewage. No one will claim that diluted sewage is wholesome to drink. Therefore while it is not claimed that the large numbers of bacteria found in some samples of ice are in themselves necessarily injurious, yet their presence in such numbers indicates danger.

On the basis of the fact that bacteria live on, and in a sense eat up the organic matter of sewage present in water, it might be said that the more bacteria present the better it is for the water. This is true; but so long as there remain large numbers of active, living bacteria in the water, this fact of itself is an indication that there still remains undevoured sewage. Besides this it matters little whether or not a water was *getting* purer just before it formed ice; if there exists in it the germs of typhoid fever, it is unfit for use.

Another argument that might be advanced is this: If it is true that typhoid bacilli are present in market ice, why are not these diseases more common among those classes that habitually use ice than among those that do not?

In answer to this it may be said that from various sources the poorer classes have more opportunities for becoming inoculated with typhold fever than the rich, though the latter are, as a rule, more vulnerable to the attacks of the microbe. Again, it is not claimed that all cases of typhoid fever or any other disease is *always* communicated by germs contained in ice, but it *is* claimed that a certain proportion of cases are produced in this way.

Another question that will be asked by the sceptical is this: If germs of typhoid fever exist in ice, why does not every one who uses ice sooner or later get these diseases?

To this the answer is that every one does not get this malady, any more than every one exposed to the contagium of scarlet fever contracts the latter disease. Every one is not equally susceptible, and the susceptibility, or rather vulnerability, of any individual may vary greatly at different times. It is no uncommon thing to see among the poorer classes a child suffering with diphtheria while surrounded by its little brothers and sisters, who, perhaps, sleep in the same room, yet do not contract the disease. Yet to say from this that it would not be dangerous for other children to breathe the air of the room, and that the germs of diphtheria did not float in the room, would be an unjustified statement. To say that all the hundreds of people in any given town have been consuming ice from a certain source for years, yet the entire population is not disseminated, is no argument that the ice is not contaminated and spreading disease. In all such towns vulnerable individuals are constantly getting typhoid fever and dying from it, while their friends are wondering where they could have contracted the disease. Many such people, if one were to suggest the possibility of water or ice being the vehicle for the conveyance of the germs, would scoff at the idea and say that such a thing was impossible, for other people had been using the same ice, yet did not contract the disease. These persons forget that it takes both a predisposing and an exciting cause to produce a malady; that there are many accessory circumstances necessary to the inoculation of a zymotic disease, and that it is only in vulnerable individuals that such inoculation is possible.

As before mentioned, I found typhoid fever germs in one sam-

ple of ice. Apropos of this it may be asked: Did every one using that ice become infected?

Definite data on this point were difficult to obtain, yet I found eleven people who had used a great deal of ice from the polluted pond. They were the members of the family of the owner of the pond and a neighbor's family. Out of these eleven people, *four contracted typhoid fever.* The fact that only four of the eleven were inoculated with the disease might seem to contradict the opinion that the ice could have been the means of carrying the specific typhoid germ. But it must be remembered that, as I have already stated, every one exposed to the germ does not become infected; and, further, as I have also stated, to say that because a certain sample of ice containing typhoid fever germs does not produce the disease in every one partaking of such ice, is as foolish a statement as it would be to declare that diphtheria is not contagious because every one who goes into the room of a diphtheritic patient does not get diphtheria. Some individuals are vulnerable to the attacks of certain species of microbes. Some individuals are vulnerable at one time and not at another. It is probable that at one time a person resists the onset of pathogenic bacteria, whereas at another time the same bacteria would gain a firm foothold and produce disease, perhaps death. Again, typhoid bacilli might be present in the ice from one portion of a body of water and not in another; for unless the water were much agitated they would not, under ordinary circumstances, scatter widely, unless they were proliferating rapidly.

Still another argument that might be advanced to show that there is no danger in a polluted ice supply, is the fact that while the period of greatest ice consumption is during the summer months, typhoid is not very prevalent till later. But it should not be forgotten that typhoid does occur in summer time, and that ice water is consumed in the fall. Further, it is not, by any means, claimed that *every* case of typhoid is the result of polluted ice.

I have spoken at some length on these matters, with necessarily some repetition, because on former occasions all these arguments have been brought forward to demonstrate that bacteriology is a humbug.

To recapitulate: No one acquainted with the data, at this late day doubts that typhoid fever is due to a specific germ. If germs cause the disease, and these germs are present in water as a liquid, and they are not killed or rendered inactive when that water solidifies, then but one conclusion is rationally possible, and that is that there is danger in a polluted ice supply.

Next comes the question: If market ice, or a portion of it, is polluted to a dangerous extent, what are we going to do about it?

The chief thing to be done is to prevent the pollution. To go into the details of how this should be done would be out of place here, involving, as it does, the whole question of pure water supply; but I would like to give the gist of some points suggested in this connection by that eminent bacteriologist, Prof. T. Mitchell Prudden, together with some further suggestions.

In the first place, the remedy is not in a diminished consumption of ice, but in measures looking to purer ice supply. Ice harvesters and boards of health (who ought to have police powers) should realize and act upon the evident necessity of careful inspection and of various safeguards against possible contamination; which contamination may result from pollution of the water of the pond, lake or river by the influx of sewage, presence of dead animals, proximity to burial grounds, or by any other means by which animal matter, animal products, or decomposing vegetable matter may reach the body of water at any time of year. Another manner of ice pollution is the flowing on its surface, and freezing there, of surface water. The time will come when sewage and dead animals will be disposed of in some way other than depositing them in our down-stream neighbor's drinking water. Until that time shall have arrived, and even thereafter, there is much that may be done by the individual consumer.

If every one was fully impressed with the necessity of the careful and thorough disinfection of the discharges of patients affected with infectious diseases, and especially typhoid fever, it would strike at the root of the evil. The slipshod way in which this is usually done, if done at all, amounts to little less than criminal carelessness, considering the manifold dangers lessened by thorough disinfection. Every family could advance the general welfare of the

community by attention to this matter; but probably it will never be done as it should be until the health authorities, with plenty of money at command, and with police powers, take hold of it.

Careful personal inspection of the source of one's ice supply, where this source is positively known, might serve to indicate its probable degree of purity.

Since "snow" ice and bubbly ice contain enormously greater numbers of bacteria than that which is transparent, it is advisable to use the former only for purposes where it does not come into contact with food or drink. Indeed, considering everything, it would be best not to allow ordinary market ice to touch anything which is to enter the human alimentary canal without subsequent cooking.

In view of the facts of the case, it is urged that it is folly to boil and filter water and then add ice to it. Water can be kept at 40° F. (which is as cold as is wholesome) by having a cooler so constructed as to surround the water to be cooled with ice, yet not coming in contact with it. Such a cooler may be made by any tinsmith, or readily improvised by any one. Many small family refrigerators are constructed so as to cool water in this way. For those who must have ice in their water, milk, tea, etc., there remains but one way, so far as I know, that is absolutely safe, under the present conditions of ice supply, and that is the artificial freezing apparatus. In this, filtered and boiled or distilled water may be used. Distilled water is readily and cheaply procurable wherever there is a steam escape pipe. From it the steam may be collected as it condenses, and will be found pure where a non-volatile boiler compound is used.

This matter of bacteria in ice should not be regarded as sensational, nor as recommending the abandonment of ice in connection with food and drink, nor as a crusade against the ice companies. The latter, as a class, are probably more painstaking in their attempt to secure a pure article than many other merchandisers. They usually reject snow ice, because it is not so good for general use; they also reject that containing manifest impurities, as mud, leaves, grass, etc. Further than this, general knowledge does not enable them to go.

*Literature.*

Prudden, Med. Record, Vol. XXXI, p. 341. Pictet et Yŭng, Compt. Rend., T. 98, p. 467. Gautier, Bull. Soc. chem., Vol. XLII, p. 146. Miquel et Benoist, Bull. Soc. Chem., Paris, Vol. XXXV, p. 552. Coleman and McKendrick, Scientif. Amer. Sup., p. 8091, 1885. Gardner, N. Y. State Board of Health Rep., 1886. Fraenkel, Zeitschrift f. Hygiene, Bd. 1, heft 2, p. 302. Pengra, Twelfth An. Rep. Secy. Mich. State Brd. Health, 1884, p. 79. Cohn, Beitrage zur Biologie der Pflanzen, Bd. 1, heft 2, p. 221.

The first of these is the authority for some of the general statements made in this paper.